

***Ophiotaenia oumanskyi* sp. n. (Eucestoda: Proteocephalidea),  
a parasite of *Lepidobatrachus laevis* Budgett, 1899  
(Anura: Leptodactylidae) from Paraguay**

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***Ophiotaenia oumanskyi* sp. n. (Eucestoda: Proteocephalidea), a parasite of *Lepidobatrachus laevis* Budgett, 1899 (Anura: Leptodactylidae) from Paraguay.** - A new species of *Ophiotaenia*, *O. oumanskyi* sp. n., is described from the intestine of the frog, *Lepidobatrachus laevis* (Anura: Leptodactylidae), from Paraguay. Among the 10 species of *Ophiotaenia* found in anurans of the Neotropical Region, only *O. bonariensis* Szidat & Soria, 1954 and *O. ecuadoriensis* Dyer, 1986 possess an apical organ, whereas it is absent in the 8 other species. *O. bonariensis* differs from *O. oumanskyi* by the total length of the strobila (400-500 mm versus 50-96 mm) and by the number of testes (120-140 versus 85-119). *O. ecuadoriensis* differs of *O. oumanskyi* by the total length of strobila (29 mm versus 50-96 mm), by the position of the vagina to cirrus-sac (posterior versus anterior and posterior) and by the diameter of the embryophore (23-26 versus 30). *Proteocephalus bufonis* Chandra & Gupta, 2007 becomes *Proteocephalus chandrae* nom. nov. (to avoid homonymy with *Proteocephalus bufonis* Vigueras, 1942). *Proteocephalus chandrae* nom. nov. is transferred to *Ophiotaenia* and becomes *Ophiotaenia chandrae* n. comb.

**Keywords:** New species - Proteocephalidae - *Ophiotaenia chandrae* nom. nov.

## INTRODUCTION

The cestodes of the order Proteocephalidea Mola, 1928 are parasites of fresh-water fishes, amphibians, reptiles and marsupials (Schmidt, 1986; Rego, 1994, Cañeda-Guzmán *et al.*, 2001), with the highest species richness in pimelodid fishes in the Neotropical Region (de Chambrier & Vaucher, 1999; Rego *et al.*, 1999, de Chambrier *et al.*, 2006). In contrast, amphibians are scarcely represented as the definitive hosts of proteocephalidean cestodes (de Chambrier *et al.*, 2006; Marsella & de Chambrier, 2008). During a herpetological survey in Paraguay between 1979 and 2002 conducted by the Geneva Natural History Museum, proteocephalidean tapeworms

belonging to *Ophiotaenia* La Rue, 1911, were found in a leptodactylid frog *Lepidobatrachus laevis*. Since this cestode differs from all 24 species of the genus described from amphibians in the world, it is described as a new taxon herein.

## MATERIALS AND METHODS

One specimen of *Lepidobatrachus laevis* Budgett, 1899 examined was killed by immersion in a 1% MS 222 solution (Methanesulfonate salt, Sigma, No A-5040) and immediately dissected. The digestive tract was fixed with hot 4% neutral formalin and subsequently stored in 75% ethanol. Strobila was stained with Mayer's hydrochloric carmine, dehydrated in a graded series of ethanol, cleared in eugenol (clove oil), and mounted as permanent preparations in Canada balsam. For histology, pieces of strobila were embedded in paraffin wax, transversely sectioned at 12–15  $\mu\text{m}$  intervals, stained with Weigert's hematoxylin and counterstained with 1% eosin B (acidified with five drops of pure acetic acid for 100 ml solution) following recently updated protocols (see de Chambrier, 2001; Oros *et al.*, 2010). Eggs were studied in distilled water. The specimens have been deposited in the helminthological collection of the Natural History Museum, Geneva, Switzerland (PLAT). All measurements are given in micrometres unless otherwise indicated. For two-dimensional measurements, length is given before width. Amphibian classification and authorities follow Amphibian Species of the World 5.5 (Frost, 2011). Abbreviations used in descriptions are as follows: x, mean; n, number of measurements; RSO, ratio of the width of the ovary to the width of the proglottis; PGP, position of genital pore expressed as percentage of its position to the proglottis length from the anterior margin; RSCS, relative size of the cirrus-sac expressed as percentage of its length to the width of the proglottis; CV, coefficient of variation. Museum abbreviations used are as follows: MHNG, Geneva Natural History Museum, Invertebrate Collection (PLAT), Geneva, Switzerland.

## RESULTS

### *Ophiotaenia oumanskyi* sp. n.

Figs. 1-8

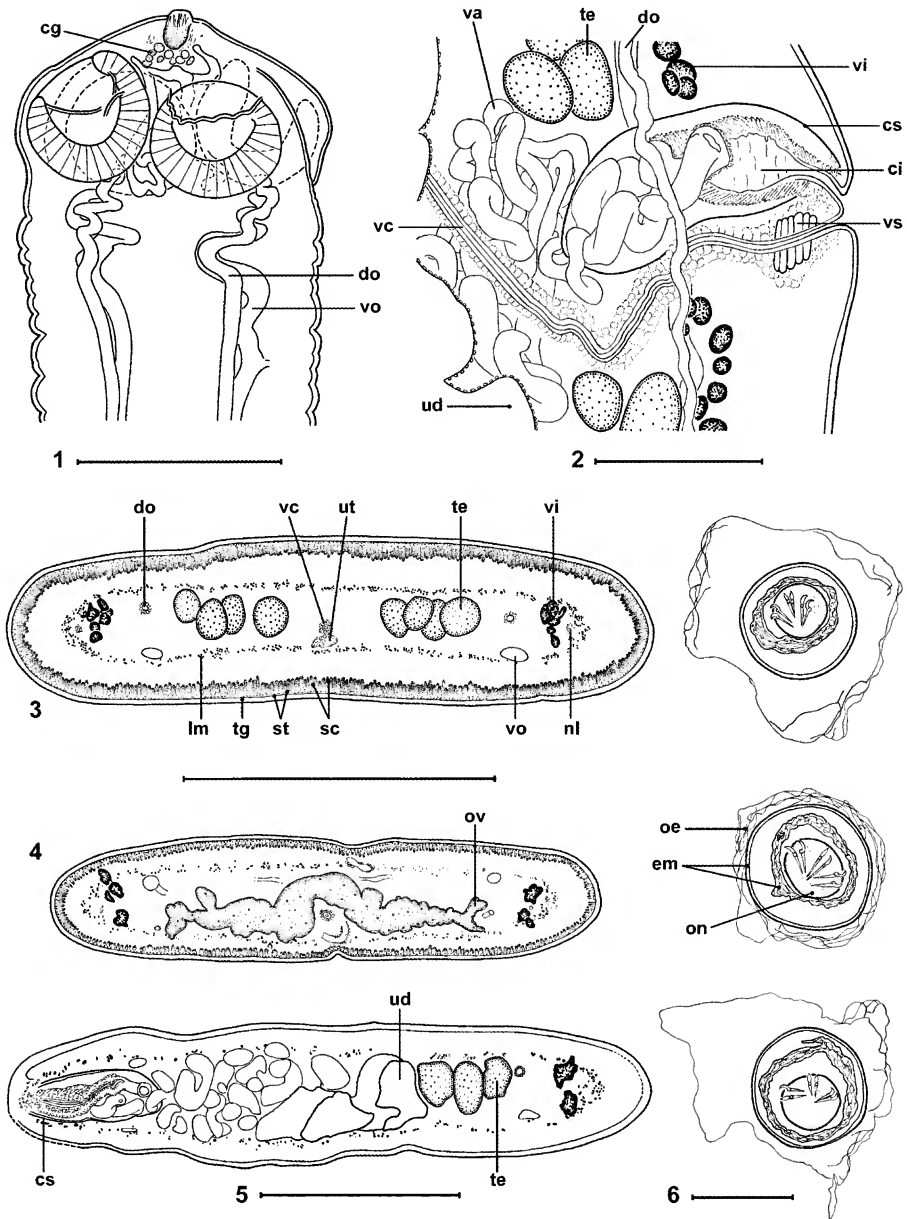
TYPE MATERIAL: Holotype MHNG-PLAT-62560, 1 whole mounted slide. – Paratype 1, MHNG-PLAT-82004, 1 whole mounted slide. – Paratype 2, MHNG-PLAT-82005, 3 whole mounted slides, 10 cross sections. All material is from the type locality and was collected by Carlo Dlouhy 4.02.2002, field number Py 9044.

DESCRIPTION (BASED ON THREE ENTIRE SPECIMENS): Proteocephalidae, Proteocephalinae. Large-sized worms, 50–96 mm long, up to 1.23 mm wide, flattened dorso-ventrally, with last proglottides elongated. Strobila acraspedote, anapolytic, with about 150 proglottides; 101–138 ( $x = 125$ ) immature proglottides (up to appearance of spermatozoa in vas deferens), 5–9 ( $x = 7$ ) mature proglottides (up to appearance of eggs in uterus), 2–6 ( $x = 4$ ) pregravid proglottides (up to appearance of hooks in oncospheres);

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### FIGS 1-6

*Ophiotaenia oumanskyi* sp. n. from *Lepidobatrachus laevis*. (1) MHNG-PLAT-62560, holotype 1. Scolex, dorsoventral view. (2) MHNG-PLAT-82004, paratype. Cirrus-sac and vagina, dorsal view; note the presence of a vaginal sphincter. (3) MHNG-PLAT-82005, paratype. Mature proglottis, transverse section at posterior part level. (4) MHNG-PLAT-82005, paratype. Mature proglottis, transverse section at ovarian level. (5) Cross-section of gravid proglottis, at level of anterior part (6) MHNG-PLAT-82005, paratype 2. Eggs drawn in distilled water.



*Abbreviations:* cg = glandular cells, probably of exocrine type, ci = cirrus, cs = cirrus-sac, do = dorsal osmoregulatory canal, em = embryophore, lm = internal longitudinal musculature, ln = longitudinal lateral nerves, oe = outer envelope, on = oncosphere, ov = ovary, sc = subtegumental cells; st = subtegumental muscle fibres, te = testes, tg = tegument, ud = uterine diverticula, ut = uterus, va = vas deferens, vc = vaginal canal, vi = vitelline follicles, vo = ventral osmoregulatory canal, vs = vaginal sphincter. Scale-bars: 1, 5 = 250  $\mu$ m; 2 = 100  $\mu$ m, 3-4 = 500  $\mu$ m, 6 = 20  $\mu$ m.

10-17 ( $x = 13$ ) gravid proglottides. Proliferation zone, 1000-1400 long. Immature proglottides wider than long; and mature, pregravid, gravid proglottides longer than wide. Last gravid proglottides elongated (length: wide ratio 2.3-5.7).

Scolex spherical, 350-410 wide, contains numerous cells with granular inclusions in the apical region. Apical organ present, 38-56 ( $x = 43$ )  $\times$  50-65 ( $x = 54$ ,  $n = 3$ ), ratio of the width of the apical organ to the width of the scolex 14-17%. Four small uniloculate suckers, 150-170 in diameter (Fig. 1).

Internal longitudinal musculature developed (Figs 3-5), forming small anastomosed bundles of muscular fibres. Osmoregulatory canals usually situated between vitellaria and testes. Ventral canal rarely overlapping vitellaria. Ventral canals 25-50 in diameter, with secondary canals ending beneath the tegument; dorsal canals 10-15 in diameter (Figs 1, 3-5).

Testes medullary, oval, 60-75 ( $x = 70$ )  $\times$  35-45 ( $x = 40$ ,  $n = 21$ ) in diameter, numbering 85-119 ( $x = 103$ ,  $n = 21$ ,  $CV = 8\%$ ), in one or two layers, in two lateral fields between anterior margin and preovarian space, reaching to ovary (Figs 4, 7), degenerated in last gravid proglottides. Occasionally, some testes overlap uterine stem. Vas deferens coiled, thin-walled, reaching to midline of proglottis (Figs 5, 7). Cirrus-sac elongate to pyriform, thick-walled, 180-260 long, representing 20-27% ( $x = 23\%$ ,  $n = 25$ ,  $CV = 8\%$ ) of proglottis width. Cirrus occupying up to 70% of cirrus-sac length (Fig. 2).

Genital ducts passing between osmoregulatory canals. Genital atrium present. Genital pores irregularly alternating, situated at 35-61% ( $x = 41\%$ ,  $n = 21$ ,  $CV = 14\%$ ) of proglottis length.

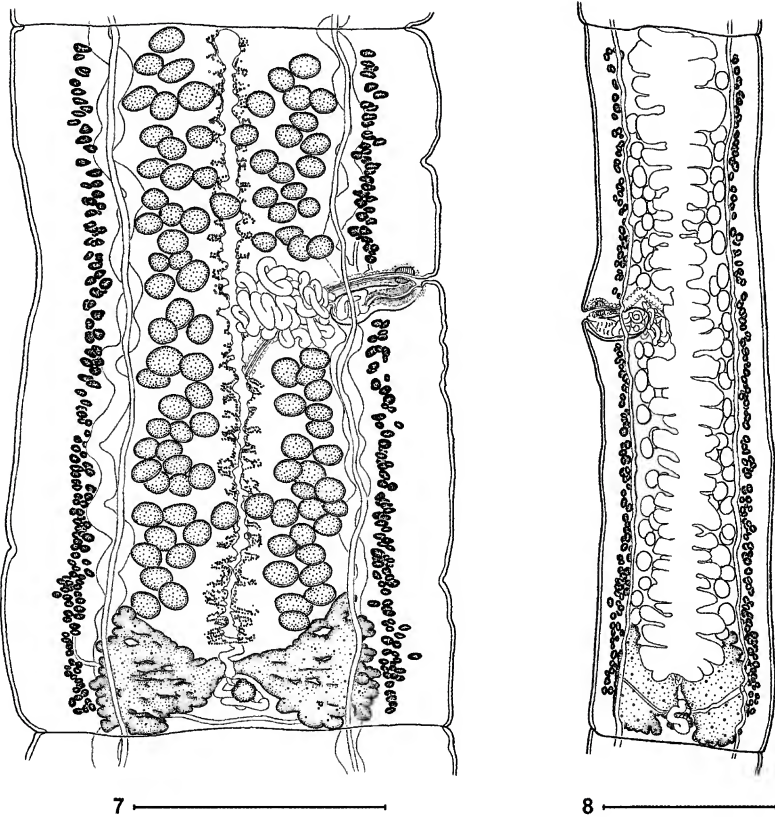
Vagina posterior (in 52% of the proglottides) or anterior (in 48% of the proglottides,  $n = 46$ ) to cirrus-sac, in proximal part lined with intensely staining cells. Muscular terminal sphincter present, 35-40 in diameter (Figs 2, 7, 8). Mehli's glands 70-105 in diameter, 9-13% of proglottis width.

Ovary medullary, bilobed, small, with follicles on ventral side 445-620 wide, occupying 61-70% ( $x = 66\%$ ,  $n = 29$ ,  $CV = 4\%$ ) of proglottis width (Figs 4, 7, 8). Ovary occupying 6.7% of proglottis surface in mature proglottis and 8.3% of proglottis surface in gravid proglottis (see Ammann & de Chambrier, 2008 and de Chambrier *et al.*, 2012 for methodology of measuring the ovarian surface).

Vitelline follicles medullary, oval to elongate, in two lateral fields, interrupted porally by vagina and cirrus-sac, reaching almost anterior and posterior margins of proglottides, occupying porally 91-97% and aporally 91-96 % of proglottis length, respectively (Figs 3-5, 7, 8).

Anlage of uterus medullary, already present in immature proglottides. Uterus with 18-25 ( $n = 13$ ) dorsolateral diverticula on each side in gravid proglottides (Figs 5, 6). Formation of uterus of type 1 according to de Chambrier *et al.* (2004): uterine stem with tubular concentration of numerous intensely stained cells and with lumen in last immature and first mature proglottides (Fig. 7). In mature proglottides, thin-walled lateral diverticula appear, with distal part lined with some intensely staining cells. At this stage, uterus occupying up to 22% of proglottis width. In pregravid proglottides, eggs completely filling uterine stem and diverticula that occupy up to 34% of proglottis width. In gravid proglottides, thin-walled digitate diverticula growing laterally,





FIGS 7, 8

*Ophiotaenia oumanskyi* sp. n. from *Lepidobatrachus laevis*. (7) MHNG-PLAT-62560, holotype, mature proglottis, dorsal view. (8) MHNG-PLAT-82004, paratype. Gravid proglottis, ventral view. Scale-bars: 7, 8 = 500  $\mu$ m.

occupying up to 72% of proglottis width, opening ventrally by several longitudinal apertures.

Eggs spherical, with thin, hyaline outer envelope, up to 55 in diameter; inner envelope consisting in two-layered embryophore, with external thick layer, 23-26 in diameter and nucleate irregular envelope, 18-21 in diameter; oncospheres 11-13 in diameter, with 3 pairs of embryonic hooks, 6-8 long (Fig. 6).

TYPE LOCALITY: Loma Plata, Filadelfia, Alto Paraguay Province, Paraguay (22°18'S, 68°18'W).

TYPE-HOST: *Lepidobatrachus laevis* Budgett, 1899 (Leptodactylidae).

SITE OF INFECTION: Intestine.

PREVALENCE: 1/1.

INTENSITY: 3 specimens.

ETYMOLOGY: The new species is named in honour of Igor Oumansky, Geneva, who facilitated our field work in South America.

DIFFERENTIAL DIAGNOSIS: The new species belongs to the genus *Ophiotaenia* because of the medullary position of gonads, the presence of four simple unilocular suckers and two lateral field testes (Freze, 1965; Schmidt, 1986; Rego, 1994).

Off the approximately 96 currently recognized species of *Ophiotaenia* parasitizing reptiles and amphibians (Schmidt, 1986; de Chambrier *et al.*, 2006; Marsella & de Chambrier, 2008; de Chambrier *et al.*, 2010, 2012), 25 species of *Ophiotaenia* parasitize amphibians and 10 of them occur in anurans in the Neotropical Region (Caribbean, southeastern Mexico, Central America and South America; Table 1) (Parodi & Widakowich, 1916; Vigueras, 1942; Wolffhügel, 1948; Szidat & Soria, 1954; Flores-Barroeta, 1955; Dyer & Altig, 1977; Dyer, 1986; Puga & Formas, 2005; de Chambrier *et al.*, 2006; Marsella & de Chambrier, 2008).

Among the 10 species of *Ophiotaenia* found in anurans of the Neotropical Region, only *O. bonariensis* Szidat & Soria, 1954 and *O. ecuadoriensis* Dyer, 1986 possess an apical organ, whereas it is absent in *O. alessandrae* Marsella & de Chambrier, 2008; *O. bonneti* de Chambrier, Coquille & Brooks, 2006; *O. bufonis* (Vigueras, 1942); *O. calamensis* Puga & Formas, 2005; *O. ceratophryos* (Parodi & Widakowich, 1916); *O. hernandezi* (Flores-Barroeta, 1955); *O. noei* Wolffhügel, 1948; and *O. olsenii* Dyer & Altig, 1977. The new species resembles *O. ecuadorensis* in the size of the scolex (350-410  $\mu\text{m}$  and 370-450  $\mu\text{m}$ , respectively), but differs in the total length (50-96 mm vs. 29 mm), the position of the vagina to the cirrus-sac (anterior and posterior vs. only posterior) and the diameter of oncospheres (23-26  $\mu\text{m}$  vs. 30  $\mu\text{m}$ ). *Ophiotaenia oumanskyi* is easily differentiated from *O. bonariensis* because the latter is very large (400-500 mm versus 50-96 mm), has a higher number of testes (120-140 versus 85-119) and the vagina is always anterior to the cirrus-sac versus anterior and posterior (see Table 1).

On the basis of the above differences, specimens found in *Lepidobatrachus laevis* are considered to represent a new species and the name *Ophiotaenia oumanskyi* sp. n. is proposed to accommodate it.

## DISCUSSION

Ammann & de Chambrier (2008) used for the first time the relative ovarian size (the ratio of the ovarian size in relation to that of the entire proglottis) as a useful character that discriminates all known species of *Ophiotaenia* parasitizing reptilian hosts in the New World from species of Palaearctic *Proteocephalus* parasitizing freshwater fishes. They found that all species of *Ophiotaenia* possess a very small ovary, with the relative size of the ovary varying between 1.9 and 5.5%, whereas that of *Proteocephalus* species is much larger (relative size 13.0-19.7%) (see Table 2 in Ammann & de Chambrier, 2008). Later, de Chambrier *et al.* (2012) calculated this character for all *Ophiotaenia* spp. from reptilian hosts (66 species) and for all remaining *Proteocephalus* spp. from freshwater fish hosts (69 species).

The relative ovarian size was newly calculated for 25 species of *Ophiotaenia* from amphibians hosts (Table 2) and was found to vary between 4.5% and 10.8% ( $x =$

TABLE 1. List of species of *Ophiotaenia* from Neotropical amphibians

Parasite species	Host	Land	Number of testes	RSCS	PGP	Vagina	Apical organ	Scolex width (mm)	Total length (mm)	Uterine branches	Eggs dimensions	Testicular field
<i>Ophiotaenia alexandreae</i> Marsella & de Chambrier, 2008	<i>Hypsiobas boans</i> (Hylidae)	Ecuador	86-128	11-17%	35-53%	posterior-anterior	no	475	138	18-25 on each side	22-24	2
<i>O. bonariensis</i> Szidat & Soria, 1954	<i>Leptodactylus latrans</i> (Leptodactylidae)	Argentina	120-140		30%	anterior	yes	800	400-500	23-27 on each side	20-27	2
<i>O. bonneti</i> de Chambrier, Coquille & Brooks, 2006	<i>Lithobates vaillanti</i> (Ranidae)	Costa Rica	100-177	15-24%	15-29%	anterior	no	280-385	380	18-32 on each side	25-30	2
	<i>Peltophryne peltacephalus</i> (Bufonidae)	Cuba	141-191	24-29%?	50%	posterior?		525-625	44-112	?	?	2
<i>O. bufonis</i> (Vigueras, 1942)												
<i>O. calamensis</i> Puga & Formas, 2005	<i>Telmatobius dankoi</i> (Ceratophryidae)	Chile	34-60	20-38%	25-50%	anterior-posterior	no	225-296	45-70	9 to 19 on each side	30-33	2
<i>O. ceratophryos</i> (Parodi & Widakowich, 1916)	<i>Ceratophrys ornate</i> (Ceratophryidae)	Argentina	?	16-20%	33%?	?	no	700	380	16-20 on each side	23	1
<i>O. ecuadorensis</i> Dyer, 1986	<i>Hyla geographica</i> (Hylidae)	Ecuador	92-121	33%	?	posterior	yes	370-450	29	22-30 on each side	30	2
<i>O. hernandezi</i> (Flores-Barroeta, 1955)	<i>Rana</i> sp. (Ranidae)	Mexico	59-78	25%	17-20%	posterior	no	850	?	21-32	?	1
	<i>Calyptocephalella gavi</i>											
<i>O. noei</i> Wolffhügel, 1948	( <i>Calyptocephalellidae</i> )	Chile	200-250	?	?	?	no	410-580	420	70 on each side	22-25	2
<i>O. olseini</i> Dyer & Altig, 1977	<i>Hyla geographica</i> (Hylidae)	Ecuador	126-160	16%	50-60%	posterior	no	395-440	43	17-27 on each side	30.5	2
<i>O. oumanskyi</i> n. sp.	<i>Lepidobatrachus laevis</i> (Leptodactylidae)	Paraguay	85-119	20-26%	35-61%	posterior-anterior	yes	350-410	50-96	18-25 on each side	23-26	2

Abbreviations: RSCS = percent of the length of cirrus-sac in relation to the width of the proglottis; PGP = position of the genital pore (cirrus pore) as % of the proglottis length from the anterior margin; Vagina = position of the vagina in relation with the cirrus-sac; Testicular field = the testes are organized in one field or in two fields; Total length (mm) = the total length of the worm.

TABLE 2. Species of *Ophiotaenia* from amphibians, with data on the relative size of their ovary

Species	Host	Locality	Ovary ratio %
<i>Ophiotaenia alessandrae</i>			
Marsella & de Chambrier, 2008	<i>Hypsiboas boans</i>	Ecuador	5.6
<i>O. alternans</i> Riser, 1942	<i>Amphiuma tridactylum</i>	U.S.A.	4.8
<i>O. amphiumae</i> (Zeliff, 1932)	<i>Amphiuma tridactylum</i>	U.S.A.	6.8
<i>O. bonariensis</i> Szidat & Soria, 1954	<i>Leptodactylus latrans</i>	Argentina	6.9
<i>O. bonneti</i> de Chambrier, Coquille & Brooks, 2006	<i>Lithobates vaillanti</i>	Costa Rica	6.9
<i>O. bufonis</i> (Vigueras, 1942)	<i>Bufo peltacephalus</i>	Cuba	7.1
<i>O. calamensis</i> Pugas & Formas, 2005	<i>Telmatobius dankoi</i>	Chile	4.5
<i>O. carpathica</i> (Sharpilo, Korniyushin & Lisitsina, 1979)	<i>Triturus cristatus</i>	Ukraine	8.8
<i>O. ceratophryos</i> (Parodi & Widakowich, 1916)	<i>Ceratophrys ornata</i>	Argentina	7.9
<i>O. chandrae</i> n. comb.	<i>Duttaphrynus melanostictus</i>	India	8.6
<i>O. cryptobranchi</i> La Rue, 1914	<i>Cryptobranchus alleganiensis</i>	U.S.A.	5.6
<i>O. ecuadorensis</i> Dyer, 1986	<i>Hyla geographica</i>	Ecuador	7.1
<i>O. filaroides</i> (La Rue, 1909)	<i>Ambystoma tigrinum</i>	U.S.A.	10.8
<i>O. gracilis</i> Jones, Cheng & Gillespie, 1958	<i>Rana catesbeiana</i>	U.S.A.	7.9
<i>O. hernandezi</i> (Flores-Barroeta, 1955)	<i>Rana</i> sp.	Mexico	10.3
<i>O. loennbergii</i> (Fuhrmann, 1895)	<i>Necturus maculosus</i>	U.S.A.	5.5
<i>O. magna</i> Hannum, 1925	<i>Rana catesbeiana</i>	U.S.A.	5.4
<i>O. niuginii</i> (Schmidt, 1975)	<i>Rana arfarki</i>	Papua New Guinea	8.6
<i>O. noei</i> Wolfllugel, 1948	<i>Calyptocephalella gayi</i>	Chile	6.6
<i>O. olor</i> (Ingles, 1936)	<i>Rana aurora</i>	U.S.A.	7.4
<i>O. olsenii</i> Dyer & Altig, 1977	<i>Hyla geographica</i>	Ecuador	6.5
<i>O. oumanskyi</i> n. sp.	<i>Lepidobatrachus laevis</i>	Paraguay	6.7
<i>O. ranae</i> Yamaguti, 1938	<i>Rana nigromaculata</i>	Japan	8.9
<i>O. saphena</i> Osler, 1931	<i>Rana clamitans</i>	U.S.A.	8.4
<i>O. schultzei</i> (Hungerbühler, 1910)	<i>Pyxicephalus adspersus</i>	South Africa	21.5?
<i>O. tigrina</i> (Woodland, 1925)	<i>Hoplobatrachus tigerinus</i>	India	8.8

7.2%) (*O. schultzei* was not considered because the drawings are not suitable for taking reliable data). In species of *Ophiotaenia* from reptiles from all parts of the World except Europe, the relative ovarian size is 1.5-6.7% ( $x = 3.4\%$ ; see table 1 in Ammann & de Chambrier, 2008 and table 2 in de Chambrier *et al.*, 2012).

Based on the new data and those of de Chambrier *et al.* (2012), it is possible to distinguish four groups in all spp. of *Ophiotaenia* and *Proteocephalus* (161 species): three for *Ophiotaenia* spp. and one for *Proteocephalus* spp: (i) 3 *Ophiotaenia* species, parasites of reptiles from western part of the Palaearctic region, with relative size of ovary 9.1-12.7% ( $x = 10.3\%$ ); (ii) 63 *Ophiotaenia* species, parasites of reptiles from all regions except for the Palaearctic Region, with relative size of ovary 1.5-6.7% ( $x = 3.4\%$ ); (iii) 25 *Ophiotaenia* species parasites of amphibians, with relative size of ovary 4.5%-10.8% ( $x = 7.2\%$ ); (iv) 70 *Proteocephalus* spp, parasites of teleost fishes from all regions, with relative size of ovary 5.4-20.2 ( $x = 11.9\%$ ) (*Proteocephalus midoriensis* Shimazu, 1990, with relative size of ovary of 28.8%, is not considered because the ovary illustrated does not seem to be of typical shape – see Shimazu, 1990, Fig. 12) (see table 1 in de Chambrier *et al.*, 2012).



These data show that the relative ovarian size of all known *Ophiotaenia* spp. from anurans is higher than those of reptilian hosts, but the number of species measured remains low and more information is needed.

As observed by de Chambrier *et al.* (2006), proteocephalidean cestodes are rare parasites of amphibians. In the Neotropical region (Costa Rica, Ecuador and Paraguay), these authors found cestodes in 11 of about 200 species of amphibians and prevalence was only 0.4% to 3.0% (de Chambrier *et al.*, 2006).

*Proteocephalus bufonis* Chandra & Gupta, 2007, a parasite of *Bufo melanostictus* is preoccupied by *Proteocephalus bufonis* Vigueras, 1942 (Chandra & Gupta, 2007; Vigueras, 1942). Due to this homonymy, we propose *Proteocephalus chandrae* **nom. nov.** for *P. bufonis* Chandra & Gupta, 2007. Furthermore, this species shows the characters of *Ophiotaenia*, such as mature and gravid proglottides being markedly longer than wide (see Freze, 1965) and thus is transferred to *Ophiotaenia* as *Ophiotaenia chandrae* n. comb.

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